PURPOSE: To standardize the quality control procedures for microbiology stains; Gram-stain and acridine orange.

REAGENTS and/or MEDIA:

- BD Gram Crystal Violet
- BD Gram Iodine (Stabilized)
- BD Gram Decolorizer
- BD Gram Safranin
- Dalynn Biologicals, Acridine Orange Stain
- Thioglycollate broth (THIO)

SUPPLIES:

- ATCC organisms
- Sterile 10 µL loop
- Glass microscope slides
- Wax pencil
- Ambient air incubator

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

PROCEDURE INSTRUCTIONS:

Step	Action
Making slides to be used for stain quality control	
1	Using fresh cultures, inoculate THIO broth with 10 colonies of *Staphylococcus aureus* ATCC 29213 and 5 colonies of *Escherichia coli* ATCC 25922.
2	Incubate broth for 2 – 3 hours at 35° in the air incubator.
3	Using a wax pencil, draw a circle on each slide. Label the slide with “Slide QC” and the date prepared. Place a single layer of the slides on a tray.
4	After incubation, using a transfer pipette, place one drop of the broth suspension onto the microscope slides in the wax pencil circle.
5	Place the tray in the 35° air incubator for 2 hours to dry.
6	Place dried slides back in the slide box and label with “QC slides”.
7	Check new batch of slides for desired performance by performing Gram-stain procedure. Refer to MIC20115 – Gram Stain Procedure.

Step	Action
Performing Gram-stain quality control	
1	Gram-stain quality control is performed daily with the first run of patient slides. During the morning start-up, a QC slide will be placed on the staining rack for this purpose.
2	A QC order is auto-generated in TQC daily.
3	Stain the QC slide with the patient slide(s). Refer to MIC20115 – Gram Stain Procedure.
4	Read the QC slide before the patient's slide(s) to ensure quality of the staining.
5	Enter the results into TQC. Refer to MIC60110.

EXPECTED RESULTS:

S.aureus ATCC 29213	Gram-positive cocci
E.coli ATCC 25922	Gram-negative bacilli

Step	Action
Performing acridine orange quality control	
1	Acridine orange quality control is performed as-tested with patient slide.
2	A QC order is auto-generated in TQC when the stain is ordered for a specimen.
3	Stain a QC slide with the patient slide. Refer to MIC20100-Acridine Orange stain.
4	Read the QC slide before the patient's slide to ensure quality of the staining.
5	Enter the results into TQC. Refer to MIC60110.

EXPECTED RESULTS:

Fluorescent organisms	Acceptable

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.
- Dalynn Biologicals Acridine Orange Stain package insert, 2014.
- BD Gram Stain Kits and Reagents package insert. 2016-01.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
		Initial Release	